

Application No. 10/790,640
Amendment dated May 18, 2009
In response to Examiner's Advisory Action dated March 6, 2009

Docket No.: 103080-P04-026

AMENDMENTS TO THE CLAIMS

This Listing of Claims will replace all prior versions, and listings, of claims in the application:

1. (Currently amended) A method of ~~rejuvenating~~ isolating a cell of the same type as a mammalian primary cell, comprising:
 - a. transferring the ~~the~~ [[a]] mammalian primary cell, the nucleus from said primary cell or chromosomes from said mammalian primary cell to a recipient enucleated oocyte or enucleated egg in order to generate an embryo, wherein

the mammalian primary cell and the recipient oocyte or egg are derived from the same mammalian species, and

said mammalian primary cell is a senescent cell or a cell that is near senescence;
 - b. obtaining an inner cell mass, embryonic disc and/or stem cell using said embryo;
 - c. injecting said inner cell mass, embryonic disc and/or stem cell into an immune-compromised non-human animal to form a teratoma;
 - d. isolating said resulting teratoma;
 - e. separating the different germ layers for the purpose of identifying specific cell types;

Application No. 10/790,640

Docket No.: 103080-P04-026

Amendment dated May 18, 2009

In response to Examiner's Advisory Action dated March 6, 2009

f. isolating a cell of the same type as the mammalian primary cell, wherein the remaining number of population doublings of the isolated cell is greater than the remaining number of population doublings of said mammalian primary cell.

2. (Canceled)

3. (Previously presented) The method of Claim 1, wherein said cell isolated from said nuclear transfer teratoma has telomeres that are on average at least as long as those of cells from a same age control teratoma, wherein said control teratoma is derived from cells that are not generated by nuclear transfer techniques.

4. (Previously presented) The method of Claim 3, wherein said telomeres are on average longer than those of the cells from the same age control teratoma.

5. (Previously presented) The method of Claim 1, wherein said primary cell is a fibroblast.

6. (Currently amended) The method of Claim 1, wherein said immune-compromised non-human animal is a SCID or nude mouse.

7. (Currently amended) The method of Claim 1, wherein, prior to step (a), said mammalian primary cell is transfected with at least one heterologous gene or at least one native gene of said mammalian primary cell is disrupted ~~of step (a), prior to transfer, includes at least one alteration to its genome.~~

8. (Previously presented) A method of making a mammalian primary cell having the same genotype as a first mammalian cell which is of a different cell type, comprising:

a. transferring the nucleus from said first mammalian cell to a recipient enucleated oocyte in order to generate an embryo, wherein

the first mammalian cell and the recipient oocyte are derived from the same mammalian species, and

Application No. 10/790,640
Amendment dated May 18, 2009
In response to Examiner's Advisory Action dated March 6, 2009

Docket No.: 103080-P04-026

said first mammalian cell is a senescent cell or a cell that is near senescence;

b. obtaining an inner cell mass, embryonic disc and/or stem cell using said embryo;

c. injecting said inner cell mass, embryonic disc and/or stem cell into an immune compromised animal to form a teratoma;

d. isolating said resulting teratoma;

e. separating the different germ layers for the purpose of identifying specific cell types;

f. isolating a cell of a different type than the first mammalian cell, wherein the telomeres of said isolated cell are at least as long as the telomeres of a cell from a same age control teratoma, wherein the control teratoma is derived from cells that are not generated by nuclear transfer techniques.

9. (Canceled)

10. (Previously presented) The method of Claim 8, wherein said first cell is a fibroblast.

11. (Original) The method of Claim 8, wherein said primary cell is of a type selected from the group consisting of smooth muscle, skeletal muscle, cardiac muscle, skin and kidney.

12. (Original) The method of Claim 8, further comprising growing said cell of a different type in the presence of growth factors to facilitate further differentiation.

13. (Canceled)

Application No. 10/790,640

Docket No.: 103080-P04-026

Amendment dated May 18, 2009

In response to Examiner's Advisory Action dated March 6, 2009

14. (Currently amended) The method of Claim 8, wherein, prior to step (a), the genome of the first mammalian cell has been transfected with at least one heterologous gene or has had at least one native gene disrupted ~~of step (a), prior to transfer, includes at least one alteration.~~

15. (Original) The cell isolated by the method of Claim 8.

16. (Original) The tissue isolated by the method of Claim 13.

17-20. (Cancelled)

21. (Currently amended) A method of performing compound genetic manipulations in a mammalian primary cell, comprising, ~~rejuvenating said mammalian primary cell~~ between genetic manipulations, using nuclear transfer of the mammalian primary cell, the nucleus from said primary cell or chromosomes from said mammalian primary cell into a recipient mammalian enucleated oocyte, wherein said mammalian primary cell is passaged to a senescent or near-senescent state prior to nuclear transfer, and wherein said mammalian primary cell and said recipient mammalian oocyte are derived from the same mammalian species, and wherein the cell resulting from the nuclear transfer has an increased number of possible population doublings remaining as compared to the mammalian primary cell.

22. (Currently amended) A method of performing compound genetic manipulations in a mammalian primary cell, comprising, ~~rejuvenating said mammalian primary cell~~ between genetic manipulations, using nuclear transfer of the mammalian primary cell, the nucleus from said primary cell or chromosomes from said mammalian primary cell into a recipient mammalian enucleated oocyte, wherein said mammalian primary cell is induced into a senescent-like or near-senescent-like state prior to nuclear transfer, and wherein said mammalian primary cell and said recipient mammalian oocyte are derived from the same mammalian species, and wherein the cell resulting from the nuclear transfer has an increased number of possible population doublings remaining as compared to the mammalian primary cell.

Application No. 10/790,640
Amendment dated May 18, 2009
In response to Examiner's Advisory Action dated March 6, 2009

Docket No.: 103080-P04-026

23. (Currently amended) The method of Claim 21, whereby said method rejuvenation results in an embryonic cell that has telomeres at least as long on average as a same age control embryonic cell.

24. (Currently amended) A mammalian primary cell produced ~~that has been genetically altered~~ according to the method of Claim 21.

25. (Currently amended) A method of making a genetically altered mammalian non-human animal ~~having the same genotype as the cell of Claim 24~~, comprising

a. providing a primary mammalian cell that has been genetically altered, and performing nuclear transfer by transferring the nucleus of the mammalian primary cell, the nucleus from said primary cell or chromosomes from said mammalian primary cell into a recipient mammalian enucleated oocyte, wherein said mammalian primary cell is passaged to a senescent or near-senescent state prior to nuclear transfer, and wherein said mammalian primary cell and said recipient mammalian oocyte are derived from the same mammalian species, and wherein the cell resulting from the nuclear transfer has an increased number of possible population doublings remaining as compared to the mammalian primary cell;

b. transferring the nucleus of the cell resulting from step (a) said mammalian primary cell of claim 24 into a recipient enucleated oocyte, wherein said mammalian primary cell and said recipient oocyte are derived from the same mammalian species [[,]];

c. [[b.]] generating an embryo or embryonic stem cell from said nucleated oocyte [[,]];

d. [[c.]] introducing said embryo or embryonic stem cell into a recipient mammalian female, wherein said recipient female is the same mammalian species as said embryo or embryonic stem cell [[,]]; and

Application No. 10/790,640
Amendment dated May 18, 2009
In response to Examiner's Advisory Action dated March 6, 2009

Docket No.: 103080-P04-026

e. ~~[[d.]]~~ allowing said embryo or embryonic stem cell to fully develop such that said female delivers a newborn animal having the same genotype as the cell resulting from step (a) said cell of claim 24.

26. (Canceled)

27. (Currently amended) A method of re-cloning a cloned non-human mammalian animal using nuclear transfer techniques, the method comprising:

- a. transferring a nucleus of a donor cell from said cloned mammalian animal into a recipient enucleated oocyte, wherein the recipient oocyte is derived from the same mammalian species as the cloned mammalian animal;
- b. generating an embryo or embryonic stem cell from said nucleated oocyte;
- c. introducing said embryo or embryonic stem cell into a recipient mammalian female, wherein said recipient female is the same mammalian species as said embryo or embryonic stem cell; and
- d. allowing said embryo or embryonic stem cell to fully develop such that said female delivers a newborn animal having the same genotype as said donor cell, wherein the donor cell used to supply the nucleus of the re-clone is a cell that is senescent or near senescence.

28. (Currently Amended) The method of Claim ~~[[25]]~~ 27, wherein said re-cloned animal has been genetically altered with respect to the cloned animal.

29. (Previously presented) A method of making a re-cloned inner cell mass, blastocyst, teratoma embryo, fetus or animal containing at least two genetic modifications, comprising:

- a. obtaining a primary cell from a mammalian animal of interest,

Application No. 10/790,640
Amendment dated May 18, 2009
In response to Examiner's Advisory Action dated March 6, 2009

Docket No.: 103080-P04-026

- b. making a first genetic modification to said primary cell by inserting heterologous DNA and/or deleting native DNA,
- c. allowing said genetically modified primary cell to multiply to senescence or near-senescence,
- d. using a first genetically modified senescent or near-senescent cell as a nuclear donor for nuclear transfer to an enucleated oocyte or an enucleated fertilized egg, wherein the enucleated oocyte or the enucleated fertilized egg is derived from the same species as the mammalian animal of interest,
- e. obtaining a cloned inner cell mass, blastocyst, teratoma, embryo, fetus or animal having said first genetic modification,
- f. obtaining a cloned primary cell from said cloned inner cell mass, blastocyst, teratoma, embryo, fetus or animal,
- g. making a second genetic modification to said cloned primary cell by inserting heterologous DNA and/or deleting native DNA,
- h. allowing said second cloned primary cell to multiply until senescence or near senescence,
- i. using a senescent or near-senescent cloned primary cell having said first and second genetic modifications as a nuclear donor for nuclear transfer to an enucleated oocyte or an enucleated fertilized egg, wherein the enucleated oocyte or the enucleated fertilized egg is derived from the same mammalian species as the mammalian animal of interest, and
- j. obtaining a re-cloned inner cell mass, blastocyst, teratoma, embryo, fetus or animal having said first and second genetic modifications.

30. (Original) The method of Claim 29 further comprising steps where said re-cloned inner cell mass, blastocyst, teratoma, embryo, fetus or animal is again re-cloned, and wherein

Application No. 10/790,640
Amendment dated May 18, 2009
In response to Examiner's Advisory Action dated March 6, 2009

Docket No.: 103080-P04-026

a third genetic modification is made such that the further re-clone has the first, second and third genetic modifications.

31. (Original) The method of Claim 30, wherein said further re-clone is generated by nuclear transfer techniques using a senescent or near-senescent donor cell.

32. (Previously presented) The method of Claim 29, wherein said re-clone has telomeres that are at least as long on average as a same age control animal, wherein said control animal was derived from cells that are not generated using nuclear transfer techniques.

33. (Previously presented) The method of Claim 31, wherein said further re-clone has telomeres that are at least as long on average as a same age control animal, wherein said control animal was derived from cells that are not generated using nuclear transfer techniques.

34. (Original) The method of Claim 29, wherein the genetic modifications involve genes that are responsible for immunological function.

35. (Original) The method of Claim 29, wherein said animal of interest is an ungulate.

36. (Original) The method of Claim 35, wherein said animal of interest is a bovine.

37-105. (Canceled)

106. (New) The method of claim 25, wherein the genetic alteration comprises the transfection of at least one heterologous gene or the disruption of at least one native gene.